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Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) eggs as alternative food for rearing of lady beetles *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae)

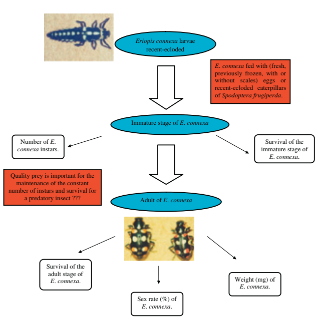
Rafael B. Silva [a,*], Ivan Cruz [b], José C. Zanuncio [a,*], Maria de Lourdes C. Figueiredo [b], Teresinha V. Zanuncio [a], José E. Serrão [c]

[a] *Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Minas Gerais State 36570-000, Brazil*
[b] *Embrapa Milho e Sorgo, Rodovia MG 424, Sete Lagoas, Minas Gerais State 35700-970, Brazil*
[c] *Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Minas Gerais State 36570-000, Brazil*

HIGHLIGHTS

- ▶ Difficulties in mass rearing can limit researches with Coccinellidae predators.
- ▶ Eriopis connexa was fed with eggs fresh, previously frozen and with or without scales or recent-hatched *Spodoptera frugiperda* caterpillars.
- ▶ The high survival of *E. connexa* fed eggs of *S. frugiperda* shows the potential of this prey to mass rear this predator.

GRAPHICAL ABSTRACT



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ABSTRACT

Eriopis connexa (Germar) (Coleoptera: Coccinellidae) is an important predator with potential for biological control of insect pests. This research evaluated the development of *E. connexa* larvae fed on fresh eggs of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) without (T1) or with (T2) scales or one-day (T3) or six-month (T4) frozen, or newly-hatched larvae of *S. frugiperda* (T5). The percentage of *E. connexa* adults was higher when larvae feeding on fresh *S. frugiperda* eggs with or without scales, or one-day frozen eggs of this prey and lower with eggs of this Lepidoptera after frozen for six months or with newly-hatched larvae of *S. frugiperda*. Duration of the larval period of *E. connexa* was 15.7, 15.8, 16.0, 17.6, and 17.3 days, respectively, with these diets. The high survival of *E. connexa* fed with eggs of *S. frugiperda* shows the potential use of this prey in the laboratory to maintain this natural enemy.

1. Introduction

The knowledge of the biology, behavior and rearing techniques of Coccinellidae (Coleoptera) predators are essential to the use of these natural enemies in biological pest control (Giorgi et al., 2009; Silva et al., 2009, 2010; Weber and Lundgren, 2009).

Semi-defined diets without animal protein, but supplemented with insects have been evaluated for Coccinellidae species (Attallah and Newson, 1966; Kariluoto, 1980; Matsuka et al., 1982; Silva et al., 2009, 2010). Alternative foods such as eggs *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Kato et al., 1999a,b; Berkvens et al., 2008; Silva et al., 2009) and eggs of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Pereira, 1997; Hoballah et al., 2004; Lundgren, 2009; Silva et al., 2010) have been used to rear Coccinellidae due to the low production cost and possibility of storage for long periods.

Suitability of preys can be estimated by the impact on ecological attributes of predators (Omkar and Bind, 2004; Obrycki et al., 2009; Singh et al., 2009). Some are highly nutritional and increase their development and reproduction rates (Kalushkov, 1998;

* Corresponding authors. Fax: +55 31 3899 2537.
E-mail addresses: rafaelentomologia@yahoo.com.br (R.B. Silva), zanuncio@ufv.br (J.C. Zanuncio).

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Kalushkov and Hodek, 2001; Omkar and Srivastava, 2003; Omkar and Mishra, 2005; Lundgren, 2009). Therefore, the food choice and sustainability of prey can help the use of these predators on pest management programs (Pervez and Omkar, 2004).

Eriopis connexa (Germar) (Coleoptera: Coccinellidae) is found in several countries of South America and it has potential to control aphid pests (Miller and Paustian, 1992; Miller, 1995). The development of *E. connexa* was better and the areas of its fat body cells was more than three times higher with *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) than with *Tetranychus evansi* (Baker and Pritchard) (Acari: Tetranychidae) (Sarmento et al., 2004). *E. connexa* showed exponential functional response (type II) with *Tetranychus urticae* (Koch) (Acari: Tetranychidae) and a sigmoidal one (type III) with *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), suggesting that this predator can adopt different strategies to the type of prey (Sarmento et al., 2007). Biological aspects of the imature stage of *E. connexa* with different artificial diets and eggs of *A. kuehniella* were studied with good results (Silva et al., 2009).

The aim of this study was to evaluate the feasibility of rearing *E. connexa* laboratory fed with eggs or newly hatched larvae of *S. frugiperda*. This information can be useful during periods of scarcity of preferred prey, allowing to rear this natural enemy with alternative food and its evaluation as biological control agent.

2. Materials and methods

The experiment was developed in the Laboratory of Insect Rearing (LACRI) of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Corn and Sorghum) in Sete Lagoas, Minas Gerais State, Brazil at 25 ± 1 °C, 12 h photophase and 70 ± 10% relative humidity. Experimental design was entirely randomized with five treatments based on the food type and four replications, each one with 10 *E. connexa* larvae. The treatments were fresh eggs of *S. frugiperda* without (T1) or with (T2) scales or one-day (T3) or six-month (T4) frozen, or newly-hatched larvae of *S. frugiperda* (T5).

The eggs and larvae of *S. frugiperda* were obtained from the mass rearing of the LACRI where adults of *S. frugiperda* are kept in metal cages lined with the type A4 bond paper as substrate for oviposition. The entire process of rearing *S. frugiperda* occurs at temperature of 25 ± 1 °C, photoperiod 12 h and relative humidity of 70 ± 10%.

The ovipositions of *S. frugiperda* offered to *E. connexa* were composed of egg masses with one or two layers with approximately 100 eggs. The scales of the eggs of *S. frugiperda* were not removed mechanically, as not all of them have scales. The ovipositions of *S. frugiperda* offered to *E. connexa* treatment with eggs frozen for one to six months were stored in Petri dishes (12 × 12 cm) in a freezer at a temperature of −20 °C. Before being offered to *E. connexa* they were thawed by gradual change of temperature, from the freezer to the refrigerator and then to the ambient conditions at room temperature when they were offered to the predator.

E. connexa larvae were obtained from the LACRI colony where this predator is fed on *A. kuehniella* eggs frozen for one week associated with the artificial diet (Silva et al., 2009). These larvae were individualized one day after hatching in 50 mL plastic cups and fed *ad libitum* according to the treatment. The treatment with newly-hatched larvae of *S. frugiperda* had 20, 30, 50 and 70 of them for each first, second, third or fourth instar larvae of *E. connexa*, respectively, being non-consumed preys removed and the consumption calculated.

E. connexa adults were sexed and weighed just after the emergence and transferred to cages (12 cm diameter and 18 cm height) closed with plastic wrap (PVC) and fed according to the treatment.

Larvae hatching to the adult emergence of *E. connexa* were observed daily with two evaluations at 08:00 A.M. and 04:00 P.M. to determine the number of instars (registered by the exuvia left after each ecdysis), duration and survival of larva, prepupa, pupa and larva to adult stages ($n = 20$), besides adult weight and sex ratio ($n = 40$). Data were submitted to variance analysis (ANOVA) and compared by Tukey test at 5% probability (Russel, 1989).

3. Results

E. connexa had four instars, in all treatments. Duration of first, second and third instars of this predator was similar between treatments, but that of the fourth was longer with *S. frugiperda* eggs frozen for six months (Table 1) like with T4 and T5.

Each *E. connexa* larva consumed 28.0 ± 5.5; 55.8 ± 5.2; 125.7 ± 9.9 and 275.9 ± 11.2 newly-hatched larvae of *S. frugiperda* during first, second, third and fourth instars, respectively, with a total consumption of 485.4 ± 14.8 larvae of this prey, during the larva stage of this predator.

Duration of *E. connexa* larva stage was longer with *S. frugiperda* eggs frozen during six months or with newly-hatched larvae of this prey (Table 1).

Table 2
Time length (days) of prepupal, pupal and larva to adult stage (mean ± standard error) of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) with different diets at 25 ± 1 °C, photophase of 12 h and 70 ± 10% relative humidity in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Prepupae*	Pupae*	Larva-adult*
T1	1.0 ± 0.0 A	3.8 ± 0.2 A	17.6 ± 0.6 A
T2	1.1 ± 0.1 A	3.7 ± 0.2 A	16.0 ± 0.1 A
T3	1.2 ± 0.1 A	3.3 ± 0.1 A	15.7 ± 0.2 A
T4	1.1 ± 0.1 A	3.6 ± 0.2 A	15.8 ± 0.8 A
T5	1.0 ± 0.0 A	3.6 ± 0.1 A	17.3 ± 0.3 A
CV (%)	16.7	9.0	5.7
ANOVA	($F = 0.8210$, g.l. = 12, $P = 0.021$)	($F = 1.3847$, g.l. = 12, $P = 0.30$)	($F = 3.1084$, g.l. = 12, $P = 0.06$)

* Means followed by the same letter, per column, do not differ (P < 0.05), by Tukey test.

Table 1
Time length (days) of each instar and larval stage (mean ± standard error) of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) with different diets at 25 ± 1 °C, photophase of 12 h and 70 ± 10% relative humidity in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	First*	Second*	Third*	Fourth*	Larval phase*
T1	3.5 ± 0.2 A	2.5 ± 0.1 A	2.6 ± 0.1 A	4.2 ± 0.4 A	12.8 ± 0.6 A
T2	3.4 ± 0.1 A	2.2 ± 0.0 A	2.4 ± 0.1 A	3.2 ± 0.2 BC	11.2 ± 0.2 B
T3	3.4 ± 0.1 A	2.7 ± 0.2 A	2.5 ± 0.2 A	2.6 ± 0.1 C	11.2 ± 0.2 B
T4	2.9 ± 0.2 A	2.6 ± 0.4 A	2.3 ± 0.1 A	3.3 ± 0.2 ABC	11.1 ± 0.7 B
T5	3.2 ± 0.1 A	2.8 ± 0.2 A	2.9 ± 0.3 A	3.8 ± 0.2 AB	12.7 ± 0.4 A
CV (%)	10.3	18.2	14.1	12.7	7.5
ANOVA	($F = 1.7277$, g.l. = 12, $P = 0.21$)	($F = 1.0042$, g.l. = 12, $P = 0.44$)	($F = 1.6002$, g.l. = 12, $P = 0.24$)	($F = 7.6797$, g.l. = 12, $P = 0.0026$)	($F = 3.8733$, g.l. = 12, $P = 0.0303$)

* Means followed by the same letter, per column, do not differ (P < 0.05), by Tukey test.

Table 3
Survival (%) of larval, prepupal, pupal and larva to adult stage (mean ± standard error) of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) with different diets at 25 ± 1 °C, photophase of 12 h and 70 ± 10% relative humidity in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Larvae*	Prepupae*	Pupae*	Larva-adult*
T1	90.0 ± 7.1 A	87.5 ± 4.8 AB	100.0 ± 0,0 A	77.5 ± 7.5 A
T2	95.0 ± 2.9 A	97.5 ± 2.5 A	97.5 ± 2.5 A	92.5 ± 4.8 A
T3	90.0 ± 0.0 A	97.5 ± 2.5 A	97.5 ± 2.5 A	95.0 ± 2.9 A
T4	100.0 ± 0.0 A	100.0 ± 0.0 A	100.0 ± 0.0 A	100.0 ± 0.0 A
T5	57.5 ± 8.5 B	76.0 ± 5.8 B	87.5 ± 8.0 A	37.5 ± 7.5 B
CV (%)	12.3	8.1	8.3	13.2
ANOVA	(F = 9.8824, g.l. = 12, P < 0.01)	(F = 7.3086, g.l. = 12, P < 0.01)	(F = 1.6559, g.l. = 12, P = 0.22)	(F = 23.0444, g.l. = 12, P < 0.01)

* Means followed by the same letter, per column, do not differ (P < 0.05), by Tukey test.

Table 4
Sex rate (%) and weight (mg) (mean ± standard error) of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) from larvae with different diets at 25 ± 1 °C, photophase of 12 h and 70 ± 10% relative humidity in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Sex ratio (%)	Weight (mg)	
		Females*	Males*
T1	0.44 ± 0.02 A	11.0 ± 0.3 AB	7.3 ± 0,07 A
T2	0.48 ± 0.04 A	10.9 ± 0.2 A	7.7 ± 0.20 A
T3	0.61 ± 0.05 A	12.7 ± 0.3 A	7.8 ± 0.61 A
T4	0.51 ± 0.07 A	11.2 ± 0.2 A	7.9 ± 0.12 A
T5	0.62 ± 0.13 A	8.6 ± 0.1 B	5.1 ± 0.20 B
CV (%)	27.6	4.6	8.9
ANOVA	(F = 1.1375, g.l. = 12, P < 0.3850)	(F = 33.1176, g.l. = 12, P < 0.01)	(F = 13.8063, g.l. = 12, P < 0.01)

* Means followed by the same letter, per column, do not differ (P < 0.05), by Tukey test.

E. connexa prepupal stage was established when larva of this predator stopped feeding and fixed to the surfaces of the cage with its last abdominal segment. Duration of this stage ranged from one to 1.2 days, without difference between treatments (Table 2).

Survival of *E. connexa* larva stage was higher with *S. frugiperda* eggs than with newly-hatched larvae of this prey (Table 3). Survival of prepupa with newly-hatched *S. frugiperda* larvae was lower than those from other treatments, except in the T1 (Table 3).

Prey type did not affect duration and survival of *E. connexa* pupa stage (Tables 2 and 3).

Duration from larva to adult of *E. connexa* ranged from 15.7 to 17.6 days without difference between treatments (Table 4). The percentage of adult emergence of this predator was higher with larvae fed on *S. frugiperda* fresh eggs without (T3) or with (T4) scales, or eggs frozen for 24 h (T2) but lower with eggs of this Lepidoptera frozen for six months (Table 3).

The sex ratio of *E. connexa* was similar between treatments, ranging from 0.44 to 0.61 (Table 4).

E. connexa females were heavier than males in all treatments (Table 4) and individuals of both sexes were lighter with newly-hatched *S. frugiperda* larvae.

4. Discussion

Number of *E. connexa* instars was similar to that found for this predator fed on *Diuraphis noxia* (Mordvilko), *Rhopalosiphum maidis* (Fitch), *D. noxia*, *Acyrthosiphon pisum* (Harris), *M. persicae* or *Cinara atlantica* (Wilson) (Hemiptera: Aphididae) (Miller and Paustian, 1992; Miller, 1995; Oliveira et al., 2004), and for *Scymnus* (*Neopullus*) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) or *Tsuga canadensis* (L.) leaves infested by Aldegidae (Lu et al., 2002) and for *Coccinella undecimpunctata* (L.) (Coleoptera: Coccinellidae) with *Megoura persicae* (Buckton) and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) (Cabral et al., 2006).

Duration of first, second, third and fourth instars of *E. connexa* was similar to that reported for this predator with *C. atlantica* (Oliveira et al., 2004), but longer when it fed on *D. noxia* and *Rhopalosiphum padi* (Miller and Paustian, 1992). This might be due to prey type, since *D. noxia* and *R. padi* might have better nutritional quality than the prey supplied in our study.

The similar number of instars of *E. connexa* fed on eggs or newly-hatched *S. frugiperda* larvae shows that both diet are adequate for this predator, since Coccinellidae often die during the first instar with low quality prey (Michaud, 2005; Agarwala et al., 2008; Phoofolo et al., 2009; Giorgi et al., 2009). Hence, prey type is important for the maintenance of the constant number of instars for a predatory insect (Scriber and Slansky, 1981; Thompson, 1999; Nava and Parra, 2005; Phoofolo et al., 2008), and shows that prey supplied to *E. connexa* were adequate.

Longest duration of first and fourth instars of *E. connexa* suggests the need for higher nutrient storage during these stages, perhaps linked to the higher metabolic need of the next instar (Scriber and Slansky, 1981; Thompson, 1999; Agarwala et al., 2008; Phoofolo et al., 2009). The longer period of development is a mechanism that allows insects to survive inadequate nutrition during the larval stage what can extend food intake to obtain sufficient food resources to complete its development (Shafiei et al., 2001).

Shorter durations for instars of *E. connexa* with *D. noxia* and *R. padi* (Miller and Paustian, 1992) might be due to the fact that aphids are preferred preys of Coccinellidae, since food quality affects development and survival of larvae of this predator (Kalushkov and Hodek, 2001, 2004; Iskber and Copland, 2002; Obrycki et al., 2009). However, differences in morphology, behavior and nutritive constitution of prey can affect the development of these predators, as reported for *Propylea dissecta* (Mulsant) (Coleoptera: Coccinellidae) and *Coccinella septempunctata* (Omkar and Srivastava, 2003; Omkar and Mishra, 2005). Hence, the use of eggs and newly-hatched *S. frugiperda* larvae shows that this prey makes possible the development of this predator.

Higher survival of the larval stage of *E. connexa* with eggs than with larvae of *S. frugiperda* is similar to that of this predator fed on *D. noxia* and *R. padi* (Miller and Paustian, 1992) and *M. persicae* and *D. noxia* (Miller, 1995). Although the larva survival of *E. connexa* was lower with newly-hatched larvae than with eggs of *S. frugiperda*, it was similar to that of this predator fed on *A. pisum* (Miller, 1995). Low larva survival of *E. connexa* with newly-hatched larvae of *S. frugiperda* might also be due to non-nutritional factors, such as prey mobility and texture, which can difficult feeding and compromise the development of this predator. During the first instar, Coccinellidae have higher difficulty to feed on large and active prey (Phoofolo and Obrycki, 1997). Growth rates, larval survival and reproduction of Coccinellidae are associated with prey quality, which may be due to the high level of protein or to increased consumption with some species (Omkar and Srivastava, 2003; Zhang et al., 2007; Lundgren, 2009).

Fresh eggs of *S. frugiperda*, with or without scales were the best diet for *E. connexa* larvae, as shown by the shorter duration and higher survival of the larval stage of this predator. This shows that scales and chorion of eggs were not barriers for larval feeding, as reported for *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) with *Ostrinia nubilalis* eggs (Hübner) (Lepidoptera: Pyralidae) (Phoofolo et al., 2007).

Consumption of newly-hatched *S. frugiperda* larvae by *E. connexa* increased with late instars, as reported for *P. dissecta* with several species of Aphididae (Pervez and Omkar, 2004). Newly-hatched Coccinellidae larvae have reduced predation ability (Hemptine et al., 1992), low consumption rate (Ponsonby and Copland, 2000) and minor voracity, due to its small size and mobility (Pervez and Omkar, 2004), as found for *E. connexa*.

The higher consumption of *S. frugiperda* larvae by *E. connexa* in the fourth instar can be explained by increasing necessity of nutrients for pupation as reported for *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae) fed on *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae) (Stathas, 2000). The highest consumption in the fourth instar by Coccinellidae also indicates the quality of food supplied, since the final larva size determines that of the adult (Schüder et al., 2004; Phoofolo et al., 2007, 2009; Honek et al., 2008). The highest necessity of food for growth and development can explain the consumption increase in the fourth instar (Pervez and Omkar, 2004).

Newly-hatched *S. frugiperda* larvae most probably have lower nutritional value, and eggs of this Noctuidae frozen for six months might have lost part of its quality, compromising the development of *E. connexa*. However, frozen eggs can increase mass rearing of predatory insects by the possibility of storing them which would reduce the effective cost for rearing, in relation to the use of fresh preys, but freezing could alter its nutritional quality (Adams, 2000; Mohaghegh and Amir-Maafi, 2007).

Duration of prepupal stage of *E. connexa* was similar to that for *Hippodamia convergens* Guérin-Meneville and *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae) (Kato et al., 1999a,b) and *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) with *M. persicae, Megoura viciae* Buckton, *Aphis gossypii* Glover or *Aphis fabae* Scopoli (Hemiptera: Aphididae) (Iskber and Copland, 2002).

The longest duration of *E. connexa* pupa than that of this predator fed on *D. noxia* and *R. padi* (Miller and Paustian, 1992) shows variation of this stage with similar values among treatments with *C. atlantica* (Oliveira et al., 2004) or *D. noxia* and *R. padi* (Miller and Paustian, 1992; Miller, 1995). The high pupa survival in all treatments shows that the food supplied provided the nutrients needed for this predator to become adult, because this stage is critical for insect development and depends on the nutrients obtained in the immature stage (Scriber and Slansky, 1981; Thompson, 1999; Schüder et al., 2004).

Similar duration from larva to adult of *E. connexa* with *S. frugiperda* eggs, fresh or one-day frozen is important because diet inadequacy can extend the life cycle of insects (Scriber and Slansky, 1981; Thompson, 1999). The absence of some amino acids during the immature stage can increase the duration of this stage in insects (Hacker and Bertness, 1996; Bottrell et al., 1998).

Low survival of *E. connexa* fed on newly-hatched *S. frugiperda* larvae suggests that this prey has lesser nutritional value or that, for not being static, increases the foraging costs and reduced the probability of capture and energy gain for this predator (Sih and Christensen, 2001; Lemos et al., 2005; Provost et al., 2006).

Adequate development of *E. connexa* with *S. frugiperda* eggs shows that eggs of this insect in layers and with adhered scales did not avoid feeding of this predator (Bezerra et al., 2002). Eggs of this Noctuidae showed high nutritional value, compared to those of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), which are relatively small and with high nitrogen content and, hence, an adequate prey for generalist predators (Eubanks and Denno, 2000).

Prey type did not affect sex ratio of *E. connexa,* because this predator showed similar values to those with *M. persicae*, *D. noxia* and *A. pisum* (Miller, 1995) and *C. atlantica* (Oliveira et al., 2004) and to those with *H. convergens* and *C. septempunctata* (L.) (Coleoptera: Coccinellidae) or *Myzus persicae nicotianae* (Sulzer) (Hemiptera: Aphididae) (Katsarou et al., 2005).

The higher weight of *E. connexa* females than males in all treatments shows that the quantity and quality of food consumed in the larval stage can affect the weight of Coccinellidae (Omkar and Srivastava, 2003). Females of this predator can be distinguished from males by their weight, as found for *H. convergens* and *C. septempunctata* (Katsarou et al., 2005). The higher weight of *E. connexa* females with *S. frugiperda* eggs is a desirable trait since heavier ones can produce larger offspring likely found for heavier females of the predator *Podisus rostralis* (Stäl) (Hemiptera: Pentatomidae) (Zanuncio et al., 2002) and *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae) (Omkar and Bind, 2004).

Fresh eggs of *S. frugiperda* are better for *E. connexa* due to the shorter larva stages and the higher weight of this predator with this prey.

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